THE EFFECT OF ULTRA-VIOLET LIGHT UPON EARLY DEVELOPMENT IN EGGS OF URECHIS CAUPO[1]

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Introduction

During the summer of 1936 while at the Hopkins Marine Station, Pacific Grove, California, the writer observed the effect of ultra-violet radiation upon the early development in eggs of *Urechis caupo,* Fisher and MacGinitie (1928*a,* 1928*b*). The eggs of this marine worm are immature when shed, hence maturation ensues after fertilization. The present paper gives results which show the effect of ultra-violet radiation upon maturation and first cleavage, together with the effect upon the rate of these early stages. The study does not aim at a physical analysis of radiation effects, but rather, at the qualitative effects of radiation upon maturation and first cleavage.

Material and Methods

The worms were collected at Elkhorn Slough, Monterey Bay Region, California. To insure the use of gametes in best condition the usual practice was to collect animals as often as tide conditions were favorable. Experimental animals were kept no longer than three weeks. They gave a yield of eggs and sperm of high fertilization capacity throughout the entire period of the work. Quantities of gametes were removed from the worms (males and females were kept in separate aquaria) when needed. Eggs were placed immediately into dishes containing 250 cc. sea water and dry sperm were kept in covered Syracuse watch glasses.

Irradiation was by means of an Analytic Model Quartz Lamp, Hanovia, which operated on a 110-volt circuit, alternating current, 60 cycles, 5 amperes.

All handling of eggs and sperm was done at a controlled temperature of 20° C. (plus or minus 2.5° C.). For regulation of the temperature of the sea water into which the eggs were placed several water-baths were used. Within each bath a thin-walled, flat-bottomed, glass dish

[1] This work has been supported in part by a grant from the Committee on Radiation, National Research Council.

was supported. This dish held the eggs and adjacent to them was placed the bulb of a calibrated thermometer.

Conditions were standardized as much as possible. The ultra-violet lamp was allowed to burn for 10 minutes before exposures were made. During this time traces of ozone were removed and the light reached its maximum intensity. Radiations were made at a distance of 30 cm. from the source of the light and the unfertilized eggs were given non-lethal exposures to the full spectrum of the mercury arc, for periods of 30, 35, 40, 45, 50, 55, and 60 seconds.

An experiment consisted of placing eggs whose controls gave 99 per cent to 100 per cent polar body extrusion and 97 per cent to 100 per cent first cleavage, in a thin layer on the bottom of a dish filled with sea water to a depth of 2.5 mm. This dish was placed in the constant temperature bath under the ultra-violet light and the eggs were irradiated. The dish was then removed and placed in a constant temperature bath fitted to the microscope, where the eggs were observed for five minutes for activation (development of fertilization membranes) by the radiation. When no activation was found the eggs were inseminated with a standard sperm suspension (one drop of dry sperm from a capillary pipette to five cc. sea water) and allowed to develop. The eggs were examined later for the extrusion of polar bodies and for first cleavage. Counts were made of the number of eggs in 100 which extruded first and second polar bodies, and of the number of eggs in 100 which showed first cleavage. These counts were repeated 19 times for each group of irradiated eggs. Twelve sets of experiments were made so that the data given below are for 24,000 eggs at each exposure.

The time-lapse was measured also, from the time of the insemination of the eggs to the time of the beginning of the particular stage considered. The time-lapse measurements for polar body extrusion were made on the reacting half of 10 eggs in the field of the microscope at the time of observation and the criterion was that moment when the polar body was sufficiently extruded to be identified unmistakably at the periphery of the egg. In cases where eggs were irradiated for 50, 55, or 60 seconds it was not possible to measure the time-lapse for the reacting half of 10 eggs because of the small number of polar bodies which could be found at the periphery of the eggs. I therefore measured the time-lapse for individual eggs and considered the average of the measurements made in the 20 counts as the time-lapse measurement. The criterion for first cleavage was that moment when the egg was elongated unmistakably at or in one or more axes (according to the regularity of cleavage) and the cleavage furrow could be seen as a thin, shining line which was especially clearly defined with the type of

illumination used. The time-lapse was measured for the reacting half of 10 eggs in the field of the microscope, but at exposures of 50, 55, or 60 seconds when there were very few cleaving eggs it was necessary to measure the time-lapse for individual eggs and to take the average of the measurements made in the 20 counts as the time-lapse from insemination to first cleavage.

The Experiments

The extrusion of polar bodies and the first cleavage of the egg, together with the time after insemination at which these phenomena occur, are affected when the egg is irradiated before fertilization. The action

Table I

Percentage of polar body extrusion and first cleavage in eggs of *Urechis caupo* exposed to ultra-violet light for various lengths of time at a distance of 30 centimeters.

Length of Exposure (seconds)	First Polar Bodies	Second Polar Bodies	First Cleavage
30	91.3	88.9	73.1
35	78.4	73.9	57.7
40	63.8	55.1	40.7
45	31.4	18.5	14.0
50	9.5	2.8	5.2
55	4.7	1.4	4.2
60	2.3	0.68	2.5

All controls showed 99 per cent to 100 per cent polar body extrusion and 97 per cent to 100 per cent first cleavage.

The percentage is based upon the average of 12 experiments in each of which 2,000 eggs were counted.

of ultra-violet light upon these early stages in development is reported as the percentage of extrusion of polar bodies and cleavage, and the effect upon the rate of reaction is reported as the percentage increase in the time-lapse for each stage. The results are presented.

Percentage Extrusion of Polar Bodies and Percentage Cleavage in Irradiated Eggs

From the results shown in Table I it is apparent that extrusion of polar bodies and the first cleavage of the egg are suppressed when eggs are irradiated with ultra-violet light. Different exposures varying from 30 to 60 seconds, with an increase of five seconds for successive durations of exposure, produce increasing suppression of the developmental stages until the longer periods of exposure are reached when there is a practically total suppression. Average percentages for all irradiations are presented for each duration of exposure which show unmistakably

that the suppression of polar body extrusion and of first cleavage varies directly with the length of exposure. Since the data are based upon observations and counts of large numbers of eggs (the average percentages are for 24,000 eggs) the evidence is fairly conclusive. These results on the suppression of polar body extrusion confirm observations

TABLE II

Effect of ultra-violet light upon the time-lapse from insemination to polar body extrusion and to first cleavage in eggs of *Urechis caupo* exposed for various lengths of time at a distance of 30 cm. Percentage increase in time-lapse equals

$$\frac{\text{time-lapse in radiated eggs} - \text{time-lapse in control}}{\text{time-lapse in control}} \times 100.$$

Stage	Length of Exposure (seconds)	Time-lapse in Radiated Eggs (minutes)	Time-lapse in Control (minutes)	Per Cent Increase in Time-lapse
First polar bodies	30	30.0	29.8	0.67
	35	31.2	28.5	9.47
	40	32.1	28.3	13.43
	45	34.8	28.6	21.68
	50	38.3	27.5	39.27
	55	41.3	28.3	45.94
	60	42.3	28.6	47.90
Second polar bodies	30	44.3	42.3	4.73
	35	46.4	42.6	8.92
	40	47.3	42.3	11.82
	45	52.9	43.8	20.78
	50	55.6	42.1	32.07
	55	57.7	42.7	35.10
	60	59.7	42.2	40.76
First cleavage	30	69.2	67.4	2.67
	35	70.1	67.2	4.31
	40	70.3	68.0	3.38
	45	75.2	68.9	9.14
	50	82.5	69.2	19.22
	55	84.0	68.7	22.27
	60	89.2	68.5	30.22

Each time-lapse measurement represents the average time from insemination to the particular stage and is based upon data for the reacting half of 24,000 eggs observed at each exposure save 50, 55, and 60 seconds. At these exposures the average time-lapse was taken for the number of eggs in 24,000 which showed the particular stage.

of Just (1933) for eggs of *Nereis limbata*. From the protocols of this observer the evidence shows that radiation effects were so pronounced that essentially a total suppression of polar body extrusion was caused when eggs were irradiated for 60 seconds at a distance of 25.5 cm. from the lamp. Just reported similar results when eggs were given longer exposures at different distances from the lamp.

Percentage of Increase in Time-lapse from Insemination to Polar Body Extrusion and First Cleavage

The data in Table II show the effect of ultra-violet radiation upon the rate of the early developmental stages in eggs of *Urechis caupo.* Time-lapse measurements for eggs irradiated at different exposures were compared with similar measurements in controls and the relation between the time-lapse from insemination to a particular stage in both the experimental and the control eggs was given as the percentage increase in time-lapse. The data show that wherever a developmental stage was affected by radiation the reaction time of the egg (time-lapse from insemination to the stage) was retarded. At the comparatively short lengths of exposure the percentage increase in the time-lapse was small. Each increase in length of exposure caused an increase in the percentage increase in time-lapse. The steady increase in the time-lapse from insemination of the egg to a particular stage as indicated in Table II may be regarded as evidence that irradiation of eggs not only affects certain stages in development but also affects the reaction rate of the eggs.

Discussion

Suppression of polar bodies and of cleavage by such agencies as exposure to extremes of the viable range of temperature for eggs, treatment with hypotonic sea water, or subjection to the action of narcotizing substances, before or after activation, has been reported by many investigators. The data reported here suggest ultra-violet light as a most effective agent for suppression of early developmental stages.

The exact nature of the action of ultra-violet radiation is not definitely known. An important factor appears to be the extent of the absorption of radiant energy by the inner protoplasm of the egg and by the superficial layer, each of which is a site of complex reactions which underlie morphological changes and developmental processes. Evidence presented by Redfield and Bright (1921) and Just (1933) shows an alteration of the initial changes involved in the cortical reaction in the egg of *Nereis* fertilized after exposure to ultra-violet light. Another type of evidence is given by Tchahotine (1921*a*), who correlated local centers of injury produced in the peripheral layer of sea urchin eggs with permeability changes. Tchahotine (1921*b*) further pointed out the probability of the coagulation of the colloids of the superficial layer of the irradiated sea-urchin egg. While eggs of *Urechis caupo,* unlike eggs of *Nereis limbata,* extrude no jelly following fertilization and show no visible alteration of the superficial layer other than the separation of a tough, pellicle-like membrane (see Chase, 1935) from the vitellus of

the egg which develops into the fertilization membrane, it is possible that certain changes take place in the egg cortex which are affected by radiation as in the case of other species of eggs. A serious alteration of the physical and chemical properties of the peripheral layer of the eggs conceivably may be a factor in the suppression of polar body extrusion in eggs which are radiated before they are fertilized.

On the other hand, the developmental stages which have been studied are closely related to various phenomena which occur deep within the egg. Of these the viscosity changes are the most widely studied. While no attempt was made to observe the effect of radiation on such changes in eggs of *Urechis caupo*, evidences of the effect of ultra-violet radiation upon viscosity of protoplasm of other eggs is reported. Of these investigations the most significant for this particular problem are the observations on the egg of *Ascaris*, which falls in the same category with the egg of *Urechis* with respect to the time at which the egg may be fertilized. Schleip (1923), in the course of observations on the effect of ultra-violet radiation on morphological components of *Ascaris* eggs, reported increased viscosity. Similarly, Ruppert (1924) centrifuged radiated eggs in his studies on the effect of ultra-violet light upon different stages in the development of eggs of *Ascaris* and his results indicate increased viscosity. Such changes alone may be associated with marked inhibition of phenomena which underlie maturation and cleavage processes and the resulting suppression of these stages in development.

A cytological study of the irradiated eggs is being made and it may give significant evidence on the effect of ultra-violet light upon morphological changes in the eggs.

Summary

1. When unfertilized eggs of *Urechis caupo* are exposed to ultra-violet light for different lengths of time, then fertilized, polar body extrusion and first cleavage are suppressed. In addition to the suppression of these stages radiation causes an increase in the reaction rate of the egg which bears a direct relation to the length of exposure.

2. Possible factors in the suppression of these stages in maturation and first cleavage are the alteration or probable injury of the egg cortex and accompanying changes in its chemical and physical properties, and viscosity changes in the egg endoplasm which inhibit internal phenomena.

The writer wishes to express his gratitude to Dr. A. C. Giese and Mr. E. W. Lowrance, School of Biological Sciences, Stanford University, and Professor

Frank Coleman, Department of Physics, Howard University, for technical advice and assistance. He is also indebted to Dr. W. K. Fisher and the staff at Hopkins Marine Station for their many courtesies.

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